Personalized medicine - the impact on chemistry

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Abstract:

An effective strategy for personalized medicine requires a major conceptual change in the development and application of therapeutics. In this article, we argue that further advances in this field should be made with reference to another conceptual shift, that of network pharmacology. We examine the intersection of personalized medicine and network pharmacology to identify strategies for the development of personalized therapies that are fully informed by network pharmacology concepts. This provides a framework for discussion of the impact personalized medicine will have on chemistry in terms of drug discovery, formulation and delivery, the adaptations and changes in ideology required and the contribution chemistry is already making. New ways of conceptualising chemistry's relationship with medicine will lead to new approaches to drug discovery and hold the promise of delivering safer and more effective therapies.

Defined key terms

- Adverse Drug Reactions
    - Unwanted harmful responses to medicines.
- Designed Multiple Ligands
    - Single drug entity to modulate multiple targets.
- Drug repurposing
    - Aiming to use previously approved drugs for a novel therapeutic indication.
- Fixed Dose Combinations
    - Combination of two or more therapies in a single delivery system for maximum effect and adherence
- High Throughput Screening (HTS)
    - Biological screen usually in a fast, parallel format.
- Network pharmacology
    - Seeks to describe therapeutically beneficial and ADR outcomes of medicines in terms of the molecular interaction of drugs with several inter-related proteins or networks.
- Pharmacogenomics
    - Study of inter-individual drug response (efficacy/toxicity) based on genetic variation.
- Polypharmacy
    - Prescription of multiple drugs.
- Polypharmacology
    - Interaction of a small drug molecule with multiple targets.
- Polypill
    - Combination of two or more preventative therapies in a single product.
- Time Release /Sustained Release Technology
    - Formulation of a drug that allows slow release over time.

Introduction

The modern use of the term "personalized medicine" has come to prominence over the past ten years [1, 2]. An excellent definition is to be found in the 2008 United States government report on 'Priorities for personalized medicine' [3]. It describes personalized medicines as "...*tailoring of medical treatment to the individual characteristics of each patient. It does not literally mean the creation of drugs or medical devices that are unique to a patient but rather the ability to classify individuals into subpopulations that differ in their susceptibility to a particular disease or their response to a specific treatment. Preventive or therapeutic interventions can then be concentrated on those who will benefit, sparing expense and side effects for those who will not.*"

The scope of personalized medicine includes predicting individual predisposition to disease, and individualising medical interventions once a diagnosis has been made [4]. Understanding the impact of genetic variability and acquired drug-induced effects on drug handling and effectiveness in the body are key concepts when considering the application of chemistry to personalizing medicines. Sequencing of an individual's genome has recently been shown to yield clinically useful predictive information by establishing the first protocol for querying disease-specific mutation and pharmacogenomic databases [5]. Categorising patients into genetically definable subpopulations based on their response to a drug or disease susceptibility [6] allows physicians to tailor a particular treatment regimen to an individual's increased likelihood to respond beneficially and/or their reduced risk of developing adverse drug reactions [ADRs]. Individuals can be categorised in this way because of multiple heterogeneities with respect to genes, lifestyle and ethnicity [6]. Based on this information, a drug might be selected to have the best therapeutic outcome at the right dose for that individual [7]. The benefits accruing through personalizing medicines risk becoming more difficult to access by the complexity inherent in network pharmacology, a new and crucially important view of existing and emerging therapeutics. Network pharmacology seeks to describe therapeutically beneficial and ADR outcomes of medicines in terms of the molecular interaction of

drugs with several inter-related proteins or networks. This review examines in detail the opportunities presented by the confluence of these ideas to the discoverers, formulaters and prescribers of therapeutic drugs.

An ADR has been defined by Edwards and Aronson as "an appreciably harmful or unpleasant reaction, resulting from an intervention related to the use of a medicinal product, which predicts hazard from future administration and warrants prevention or specific treatment, or alteration of the dosage regime, or withdrawal of the product" [8]. Genetic factors are implicated in many ADRs [9] however development of an ADR can have other causes than the interplay of genes. These include ageing, a wide range of chronic disorders, including reduced renal or hepatic function, and effects of other drug treatments to reduce the therapeutic dose requirement of some drugs. If the need for dose reduction is not recognized by a prescriber, toxic levels of a drug and its metabolites may inadvertently be achieved, resulting in avoidable ADRs.

Pirmohamed *et al.* estimated in 2004 that around 7% of urgent admissions to hospital in the United Kingdom were caused by ADRs costing a total of £466 million [10]. In addition, during 2001-2002, of the 7% of patients admitted to hospital as a result of an ADR, 2.3% died as a result, with 72% of these ADRs being classed as avoidable. These serious ADRs were considered largely due to individuals being prescribed multiple therapies. The term ADR has replaced both "side effects" and "toxic effects" to include all unwanted effects, since a side effect can include beneficial effects from a medication. There is growing interest in the value of screening for gene product variants to support personalized medicine strategy to reduce the risk of serious ADRs.

Pharmacogenomic research is yielding increasing insight into how an individual's genomic make-up affects response to a medication [11]. Knowing which targets are responsible for a disease or for adverse effects in a particular individual will better allow physicians to combat diseases by improving drug efficacy on a personalized level [13, 14]. Currently, clinicians still largely use the "one-drug-fits-

all' approach [12] where the average cohort response to a treatment is taken to imply that the average person will experience these average effects. There is a need to shift focus from a "one-drug-fits-all" target approach to one where it becomes imperative to better understand how the numerous targets involved in disease and adverse or beneficial effects of treatment are inter-related.

The main class of enzymes associated with processing drugs and xenobiotics is the cytochrome P450 family (CYP450), which is found predominantly in the liver. There is a large scope for variation among individuals in activity of CYP450 enzymes. An individual's genetic makeup of this subset of enzymes has become a major area of interest for 'personalisation' of drug prescription and dose. A classic early example of this variation was reported by R.L. Smith, who observed that a sub-group of patients taking the now superseded anti-hypertensive medication debrisoquine experienced a large decline in blood pressure [13]. He reported that autosomal recessive impaired hydroxylation by the cytochrome P450 CYP2D6 drug metabolising enzyme in the liver was responsible. Individuals with two recessive alleles for this hydroxylating enzyme have an inherited inability to metabolise debrisoquine [14]. It is estimated that in some Caucasian populations, up to 10% of individuals may have a poor metabolising CYP2D6 genetic variant. Other clinical consequences could include reduced pain relief due to poor metabolism of the prodrug codeine to its active metabolite morphine [15] and sudden death risk cardiac arrhythmias from ECG QT prolongation with terfenadine through HERG channel activation [Figure 1]. CYP2D6 is now recognised to be involved in metabolising many currently prescribed medicines. Polymorphisms of this and other CYP450 genes can confer poor, intermediate, extensive or ultra-rapid metabolising phenotypes [16].

A similar concept now in clinical practice arises from genetic variation in activity of the purine metabolising enzyme thiopurine *S*-methyltransferase (TPMT), which metabolises the thiopurine class of drugs [17] by *S*-methylation [18]. Genetic variants in the TMPT gene may lead to reduced

inactivation of these drugs, which would result in accumulation of high levels of the active drug. This puts patients at risk of developing life-threatening myelosuppression [19]. Screening for TMPT activity before the prescription of thiopurine drugs minimises risk of this severe ADR [20]. Incidence of drug-metabolising variants differs with ethnicity [21, 22].

Genes involved in processing other drugs will become easier to identify as genotyping costs decrease. Genome Wide Association Studies (GWAS) [23] will be helpful in their identification, but to reduce false-positive and false-negative results, large studies in separate well-phenotyped populations needed as well as better understanding of heterogeneities such as age, gender, ethnicity and co-morbidity [24]. GWAS may also be limited [25] due to a lack of case controls [26] and inadequate understanding of the mechanisms of serious adverse drug reactions. A recent study has shown that many drugs share the same type of ADR-causing mechanism [27]. Identifying these multiple targets that are the root cause of ADRs through a 'chemical-protein interactome' [28] or extension of an archetypal protein network illustrated in Figure 1 would be useful both in drug development and drug use in practice. The hypothesis advanced using *in silico* work[27, 28] is allied to that found through protein complementation assays,[29] in that drugs sharing similar therapeutic effects through known targets frequently also share 'hidden' phenotypes, whether associated with ADRs or additional therapeutic benefit.

A clear example of the complexity of this approach in clinical practice is achieving effective anti-coagulation with the anti-coagulant vitamin K antagonist warfarin [30], which is inactivated by the genetically variable enzyme CYP2C9 [31]. Patients with poor metabolising variants of CYP2C9 have higher than expected levels of warfarin for a given dose [33]. Synthesis of coagulation factors II, VII, IX and X involves oxidation of the important co-factor vitamin K, which is then recycled to its reduced form by the enzyme vitamin K epoxide reductase (VKORC1) [32]. The maintenance dose of the drug is usually achieved by dose titration based on measurements of the time for a patient's

blood to clot when the coagulation factor II (pro-thrombin) is added compared to a control sample (INR: International Normalised Ratio). Warfarin prevents reduction of vitamin K by inhibiting VKORC1 [32]. Patients who already a low reducing activity genetic variant of VKORC1 will have greater then expected bioactivity of warfarin [33]. It appears that genetic variation leading to increased warfarin bioactivity is present in higher frequency amongst Caucasians than Asian populations [34]. These patients are at increased risk of serious haemorrhage such as intracranial or abdominal bleeding [35]. However if warfarin is not sufficiently clinically effective, patients can develop thrombosis [36] in the heart or arteries leading for example to stroke [37] or in veins leading to pulmonary embolism [38]. Risks of ADRs from warfarin may be reduced if pharmacogenetic testing is undertaken. However this is complex in clinical practice [39] as activity of these enzymes is also influenced by environmental factors which alter liver enzyme activity, such as other drugs [40] and alcohol [41]; secondly warfarin is highly protein-bound, so that a small degree of displacement of warfarin from binding sites in the blood stream by another highly protein-bound medicine leads to a large increase in concentration of bioactive, unbound warfarin [42]. As a result, genetic variability in CYP2CP and in VKORC1 appears to account for around one third of the variability in clinical dosing with warfarin [43].

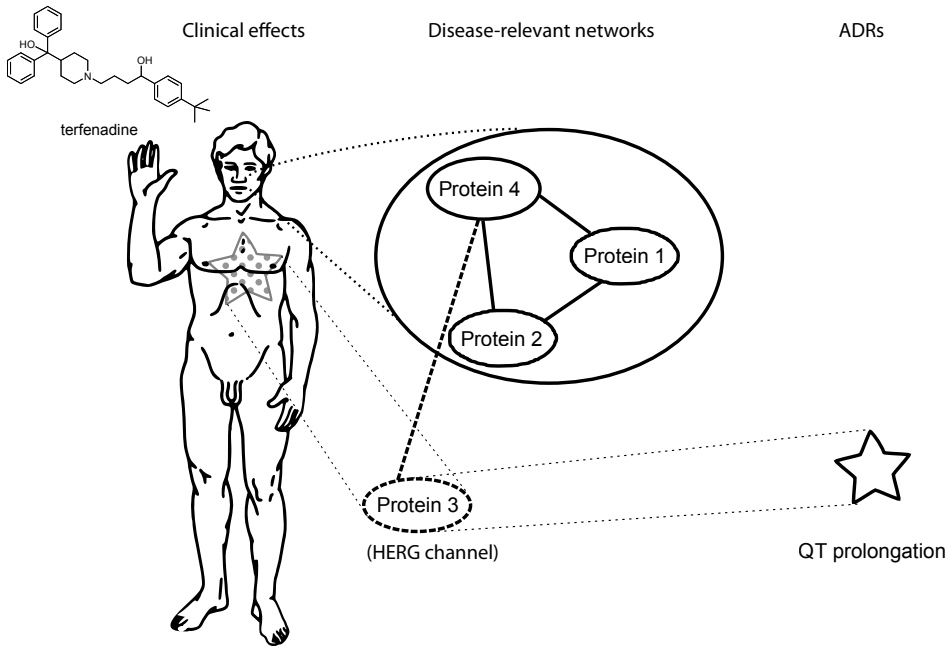

Figure 1: An example of a serious adverse drug reaction – ECG QT prolongation caused by the pro-drug terfenadine, exacerbated by inhibition of processing by CYP2D6 or by other inhibitory substances such as naringin in grapefruit juice [45]. Protein network within the large solid ellipse represents a disease relevant network with possible link to ADR-causative protein 3 indicated by dashed line. In the particular case of terfenadine, protein 3 might be the HERG channel, which is believed responsible for observed ECG QT prolongation. Graphic inspired by Figure 2 in [64], modified with permission; Pioneer 10 plaque image of man used with thanks to NASA Ames Research Center[46].

Medicinal chemists and formulation scientists of the future must be aware that in addition to genes controlling the effects of drugs, drugs may also alter gene function [7] [47]. A wide range of drugs can also alter the rate of their own metabolism, by induction or inhibition of drug metabolising enzyme activity. A key example is rifampicin (US: rifampin), a bactericidal antibiotic drug of the rifamycin group [48] used to treat tuberculosis and other serious infections. When rifampicin was

tested in a microassay in human hepatocytes to quantify mRNA expression of drug metabolising enzymes [49], there was a 3.7-fold increase in mRNA for CYP2C8 and an astonishing 55-fold increase of mRNA for CYP3A4.

A further setting where personalising medicine is important is cancer therapy, another example for which the concept of "one drug fits all" does not hold. The dose at which to prescribe anti-neoplastic agents is critical, since their therapeutic window is typically narrow and the therapeutic dose range varies due to tumour heterogeneity [50]. Use of genomic technologies to optimise the right dose for the individual, would be expected to result in increased efficacy, and a reduction in ADRs, leading to improved patient adherence.

Impact on Chemistry

A change in approach is required in order to advance personalized medicine, and for medicinal chemistry to have an impact on drug discovery programs in both academia and the pharmaceutical industry. Consideration of network pharmacology, contingent cellular pathways, and the use of emerging chemical biology technologies, such as chemical genomic tools, will facilitate individualised therapy to the benefit of the patient. Some of the challenges and benefits from this network approach are clear when considering clinical use of the anti-cancer drug trastuzumab (Herceptin®) [51]. This depended on identification of a variable treatment target: the observation that in around 30% of early stage breast cancers, there is selective expression of human epidermal growth factor receptor 2 (HER2) as the major driver for tumour growth [52]. This led to a drug discovery pathway resulting in clinical development of a new biological, a monoclonal antibody, as the treatment; and parallel development of personalized diagnostics to identify the 30% of cancer patients with HER2 who could benefit from this expensive drug, based on immunohistochemistry or fluorescence *in situ* hybridisation (FISH) detection methods applied to cancer tissue biopsies [53].

Reductionism

Within chemical biology and medicinal chemistry there has been an urgency to reduce or make simpler the interactions between a small drug molecule and a single target [54] contrasting with the integrationist approach that physiologists and more recently systems biologists have inherently built into their research approach. Embracing this integrative approach will allow chemical biologists and medicinal chemists better to understand complex biological systems, as required by network pharmacology. This is in contrast to designing maximally selective ligands to act on individual drug targets (Figure 2), an approach with notable successes, including for example the clinically licensed sumatriptan, that provides relief from migraine by selective agonist effects on the 5-HT1D receptor subtype in temporal blood vessels [55]. However, this therapy and many others do not work in all patients with the disorder, hence the need for an individualised approach.

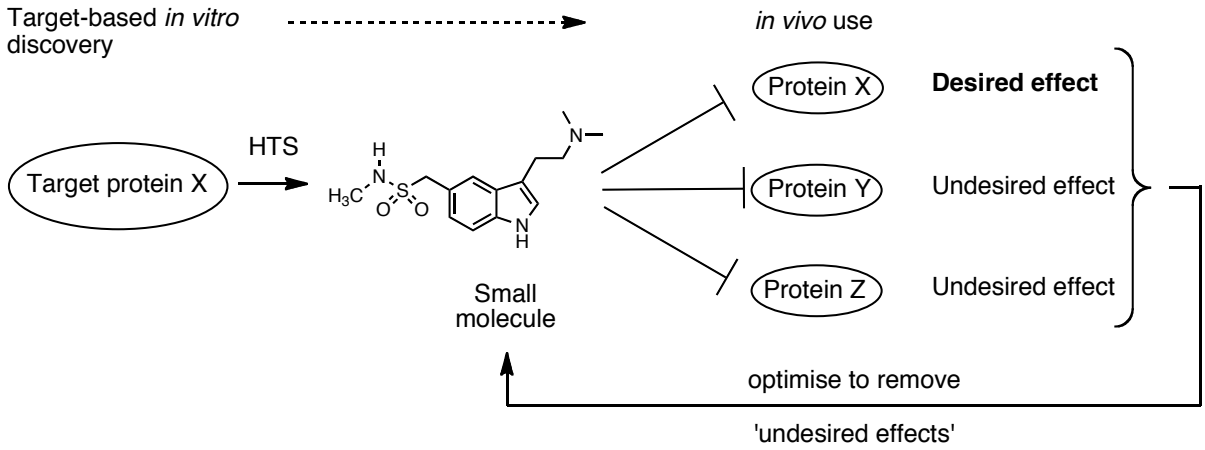

Figure 2: Single target small molecule discovery; graphic based on Figure 3 in [54], reproduced with permission.

In essence there has already been a key reductionist transition from pharmacogenetics to pharmacogenomics – the wealth of information from the genome allowing us to implicate multiple gene effects on disease susceptibility and drug metabolism. Genome-wide searches for candidate genes tend still to be carried out in a “one gene, one drug and one disease” framework in line with Paul Ehrlich’s "magic bullets" theory [56], rather than drawing on fresh approaches from chemical

biologists and medicinal chemists. Indeed, pharmaceutical companies are set to make their first loss in decades [57] with patents ending on highly target-selective drugs and, in addition, the subsequent decline in new selective drugs making it to effective therapies due to a lack of clinical efficacy and/or clinical safety. Clinical attrition figures therefore no longer support the current paradigm in producing drugs that are extremely selective; fewer drugs are making it to phase two and phase three clinical trials [57].

Network pharmacology

Systems biology has shown us that organisms exhibit phenotypic robustness [58, 59] due to cellular and organismal network complexities. Diseased states are often resistant to perturbation of a single node due to contingent mechanisms and pathways which can result in a particular patient not responding, or becoming nonresponsive to a therapy [60]. It has been shown that very selective compounds for one target may actually have a lower clinical efficacy than multi-target drugs [57]. Tackling multigenic diseases effectively, or diseases that affect multiple tissues or cell types such as diabetes and immuno-inflammatory disorders [61], typically require a physician to prescribe a mixture of monotherapies which, paradoxically, comprise a treatment regimen initially developed via rationale drug design to take out single targets. Serendipitously, several current drugs have been found to modulate a biological response by hitting multiple targets. For example it appears that cardiovascular benefits from statins may result from modulation of multiple targets in addition to reducing cholesterol levels by HMG CoA reductase inhibition [54]).

A network describes a series of interconnected nodes, where a node can be a gene or a gene product. As discussed in a review by Hopkins [62], a number of experiments support the case for multi-targeted therapeutics. A project to delete known genetic targets for drug metabolism in the mouse and profile the knockouts using phenotypic assays has shown that less than 10% of the knockouts demonstrate phenotypes that may be of use toward drug target validations. In addition, single gene knockouts exhibit little or no effect on phenotype, showing the interplay of numerous

genes in many processes, including drug metabolism. Only 19% of genes were found to be essential across model organisms and from systematic genome-wide homozygous gene deletion in yeast, 15% of the knockouts result in a 'fitness defect'. Many patients often do not respond, respond minimally or adversely to a particular therapy due to the fact that many diseases are multi-factorial and acting on a single node in a network under the current paradigm is not always sufficient [63, 64]. Already, numerous networks/pathways have been elucidated with regards to metastatic breast cancer [65] and have allowed the identification of specific markers using a protein-network based approach.

It has been suggested that network pharmacology might also be used to rationalise why many compounds fail during later phases of clinical trials [62]. Pharmacogenomics and pharmacogenetics can potentially describe why this may be true for certain subsets of the population; overlayed knowledge of network pharmacology will bring an understanding of how to meet this challenge. Drug repurposing [66] aiming to use previously approved drugs for a novel therapeutic indications will benefit in particular from this new view point. These compounds have already been extensively studied and thus enter clinical practice at a much faster rate. High connectivity amongst apparently unrelated cellular processes supports the concept of drug repurposing [67].

In order to discover possible points for therapeutic intervention whilst taking account of the robustness of biological phenotypes, an interactome or network picture is needed [68]. Methods for creating this picture and crucially, validating experimental models, link experimental and computational tools in a very direct way. In this emerging view (Figure 4), if a protein subnet is modulated without reference to certain target(s) such as protein Z this may lead to a particular ADR, whereas co-modulation maintains network stability. Cyclooxygenase inhibitors might be an example of such a circumstance with treatment increasing ADRs from upper-gastro-intestinal bleeding through interference in the mucosal barrier which may be protected by reducing gastric acid secretion by co-treatment with a proton pump inhibitor [69] (ref). Selective inhibitors of COX 2

proved unexpectedly to produce a different range of ADRs [70]. Although biochemical and prescriber knowledge has advanced, there remains unmet therapeutic need and hence medicinal chemistry opportunity as indicated in Figure 3.

Such a picture has also been deciphered for the B-lymphocyte to identify targets of molecular perturbations in a variety of non-Hodgkin's lymphomas from indirect expression data [71]. Networks in 'normal' cells and those in disease states such as cancer will be perturbed differently by therapeutics. The differential expression of cytochrome P450 enzymes leads to additional challenges and opportunities that strengthen the case for a personalized, network-centric or multi-targeted therapeutic approach [60, 67]. Thinking in the areas of polypharmacy and polypharmacology have already been helpfully linked to network pharmacology and to medicinal chemistry concepts of ligand binding efficiency [72].

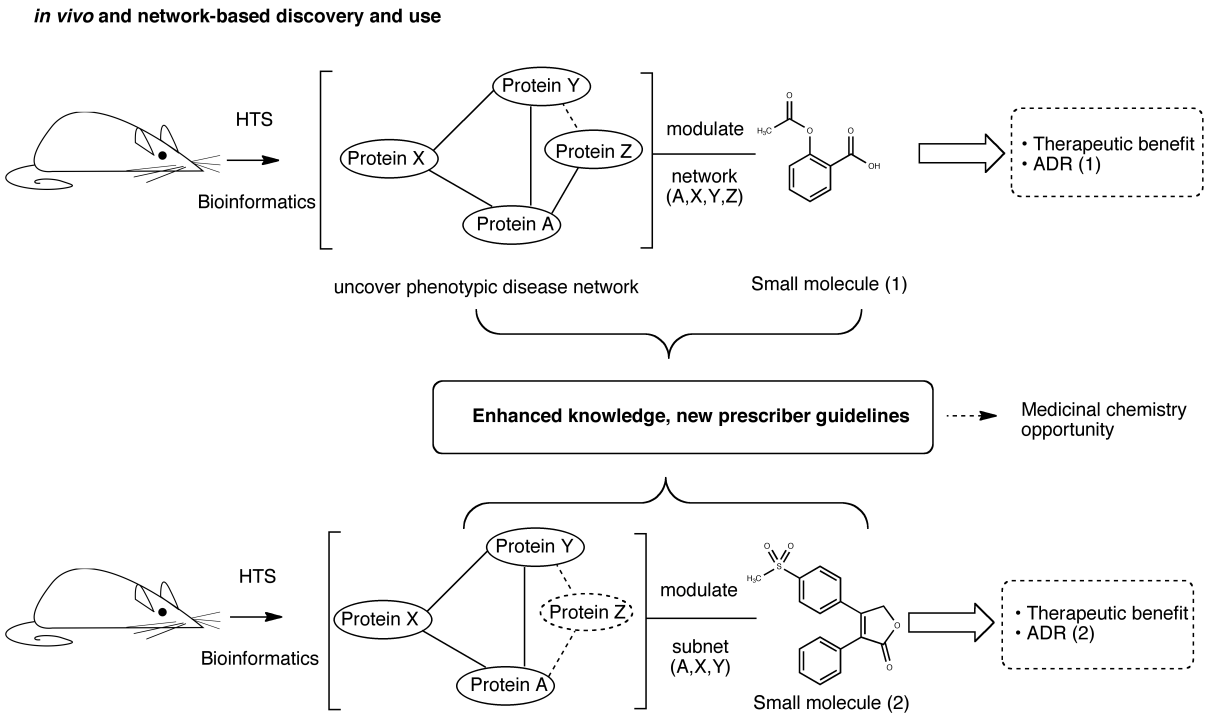

Figure 3: *In vivo* small molecule discovery approach facilitated by revealing a phenotypic disease network. Solid network edges represent a subnet of therapeutic benefit; dashed lines represent a

subnet leading to ADRs. Individual variability leads to differences in network nodes and edges through genetic polymorphism, deletions and copy number variants.

Understanding the interface between chemistry and pharmacology

Polypharmacology [73] studies the action of a single compound and its modulation of two or more molecular targets, for example the inhibition of COX isoenzymes 1 and 2 (together with other eicosanoid by non-selective non-steroidal anti-inflammatories represents an example that has only become apparent through the development of selective COX inhibitors. The goal of polypharmacology is not to increase or introduce promiscuity into a compound, rather to find compounds that have a biological effect by interacting with multiple targets known to play a key role in modulating the diseased state. Mapping these polypharmacological interactions will allow medicinal chemists to identify the best ways of providing multiple hits. We note that natural products have long been recognised as pleiotropic molecules that exhibit polypharmacology [74].

Polypharmacy [75] refers to the prescription of multiple, usually single drug target medications by a physician or their use by a patient [76] (for example Figure 4). Clinicians have found current drugs typically need to be prescribed in combinations for drugs that are extremely selective for one node when multiple nodes must actually be antagonised to see therapeutic benefit when the disease is more severe, for example in heart failure [77] and in asthma [78]. ADRs are additive as polypharmacy increases, because each additional drug has potential adverse effects and concomitant drug-drug interactions. This highlights both potential benefits and risks that may accrue with a change in drug discovery logic.

The polypill [79] is a single pill containing a combination of active ingredients to reduce the burden of taking multiple tablets. The concept was originally created for the prophylaxis of serious cardiovascular disease, with the aim of combining a range of cardioprotective drugs in a single capsule [80] to give maximum effect and reduce poor adherence associated with multiple separate

treatments. Individuals have multiple mechanisms operating within the same and different diseases. The polypill philosophy is to give 'a bit of everything' to reduce population risk of cardiovascular disease by treating the major cardiovascular risk factors: cholesterol, and diabetes and hypertension; thus protecting everyone, irrespective of genetic susceptibility to future cardiovascular problems. Whilst the polypill approach does try to take into account the variation in mechanisms among individuals within a population, and may be an attempt to personalize medicine, it does so in impersonal fashion in that it does not take into account pharmocogenetic influences on individual constituent drugs nor the increased potential for ADRs arising from failure to titrate dose or to personalise choice of agent.

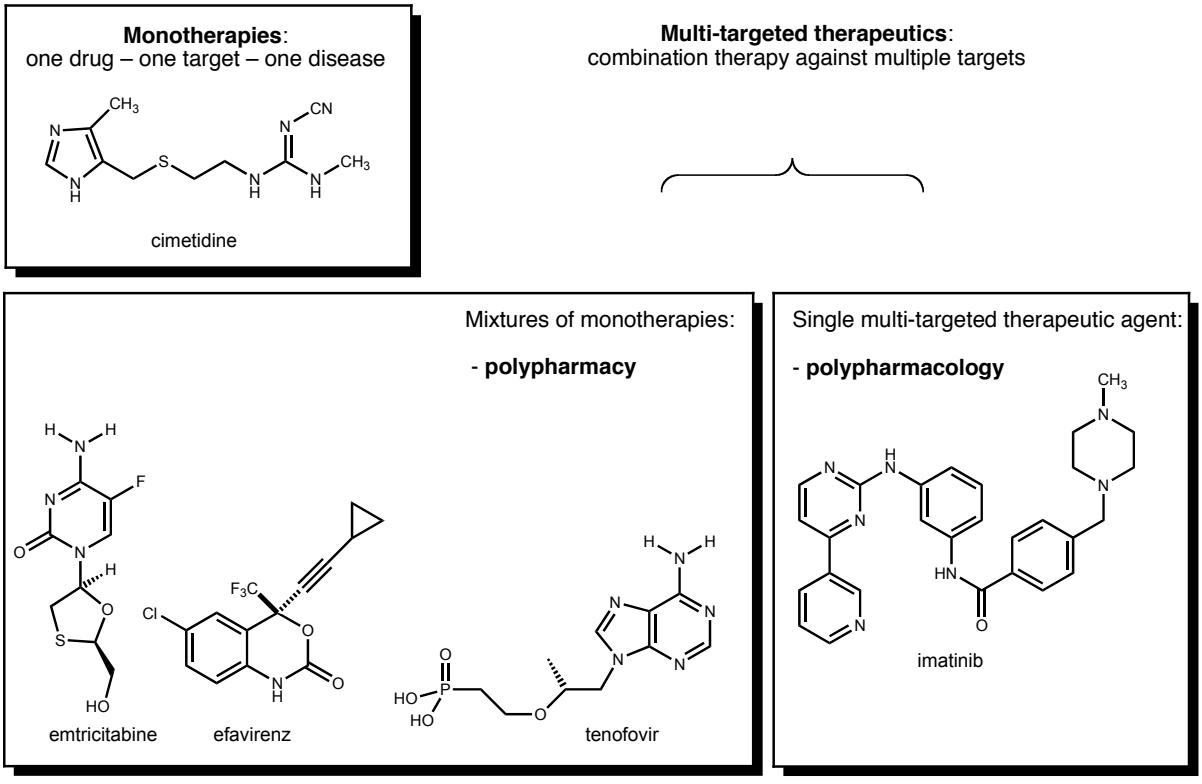

Figure 4: Examples of drugs regarded as monotherapies, mixtures of monotherapies and multi-targeted therapeutic agents [61, 81], The boundaries between these classifications are not always clearly defined and may change over time.

Towards personalized multi-targeted therapeutics

Current drug design involves approaching dysregulation of a disease-causing cellular process (resulting from genetic or epigenetic changes) and antagonising a molecular target or node central to that dysregulation. Network pharmacology aims to gain a greater understanding of dysregulation and the target(s) that are central in comparison to the healthy state in order to uncover those to modulate for optimal therapeutic benefit [82] (Figure 5).

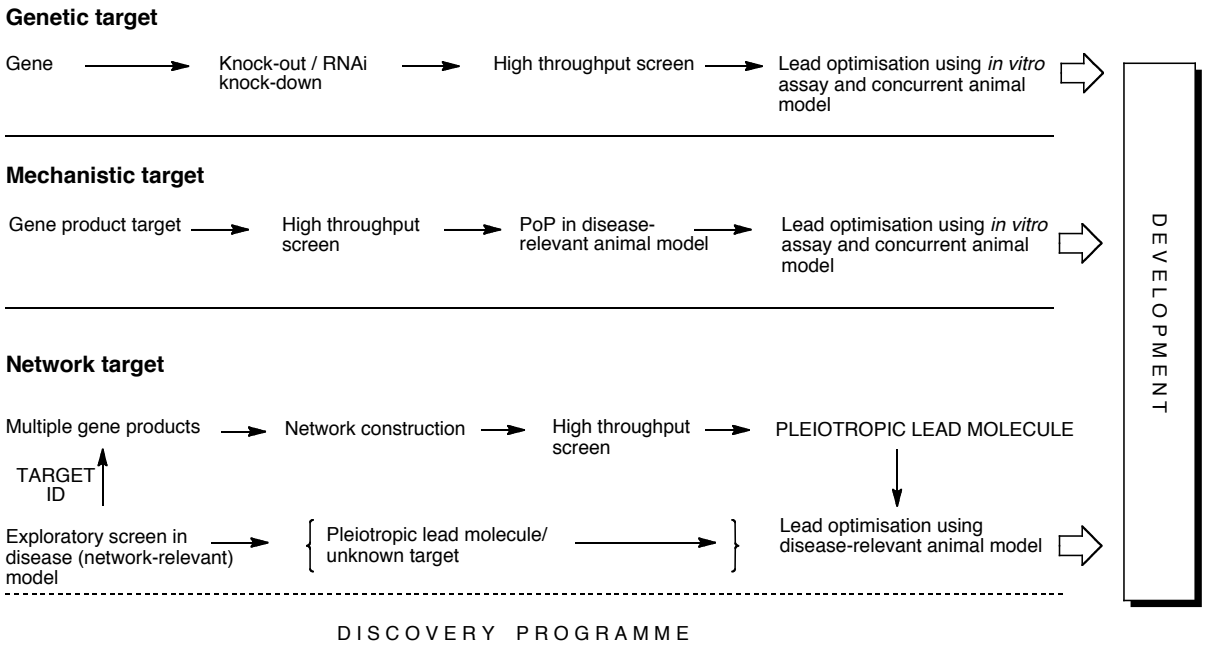

Figure 5: Comparison of lead molecule discovery in genetic target, mechanistic target and network target approaches. Parentheses indicate a classic pre structure-based design approach. Modified with permission from Figure 4 in review [83].

Personalising these combinations based on the fact that there will be genetic variation in each of our networks and nodes might be readily accomplished. Patients with the same phenotypic disease may in fact have different altered networks that might account for the difference in success or failure of treatment. Medicinal chemists and formulation scientists can respond by firstly taking on

board these changes to drug discovery ideology and then creating in effect, personalized “polypills” (by altering combinations and titrating doses).

A clear justification for this multi-targeted therapeutic approach is provided by Zimmerman *et al.* [61] using an oncology analogy in relation to tumorigenic viruses, such as human papilloma viruses and hepatitis B and C. These viruses encode proteins that block the actions of the tumour suppressor genes p53 and p105Rb [84]. By inhibiting several mechanisms that usually prevent the cell from inappropriate cell cycling and proliferation, the virus essentially ensures its survival and replication. This is an evolutionarily conserved mechanism and attacking the cell on multiple fronts allows it to survive. Multiple interventions at these nodes and others will likely be key to preventing these survival or ‘development of resistance’ mechanisms.

There are a number of advantages and disadvantages when considering combination therapies/multi-targeted therapeutics:

Mixture of monotherapies either as a polypill, co-formulated or prescribed separately:

- Advantages:
    - The ratio of each monotherapy can be titrated to account for the difference in potential node potency, presence and variability amongst individuals. You can therefore tailor hypertension medication differences between the young versus old and those from black Caribbean or African descent [85]. Genetic variability may lead to large differences in the effective quantity of each drug.
    - Achieve sequenced action[86, 87] at a specific target; e.g. for induction-remission therapy for inflammatory disease [88] or cancer, the disease response is suppressed for the initial weeks of treatment with one strategy and then a second strategy begun once initial disease severity is contained.

- Potentially a better outcome in clinical trials (*cf.* multi-targeted single agent); possible cost savings although the toxicity of the individual components must be established if not known.
- Mixture of monotherapies as a polypill improves adherence [89].

- Disadvantages
  - Both the pharmacokinetics and phamacodynamics will need to be adjusted in a co-formulation and this may not be readily predictable. A network approach may help.
  - Trials are more complex when looking at different combinations. Blinding two treatments for prescriber and patient adds difficulty. In addition, order of administration effects need to be considered where there are mechanisms in common. For example both thiazides (T) and dihydropyridine calcium channel blockers (DHP) cause increased renal water and sodium excretion. When treating hypertension, the DHP nifedipine is additive to T however T is not additive to nifedipine [90]. Problems in sequenced action might impact co-formulation in a polypill or impair patient adherence, giving scope for alternative personalizing of therapy.

Multi-targeted single agents:

- Advantages
  - Regulatory approval process for companies is very attractive as it fits well within the current business mode.
  - Improvements in patient adherence by taking one pill for a particular condition instead of many (although polypill and coformulation offer alternatives)
  - Sequenced action possible with a single agent through innovative chemistry e.g. selective delivery, controlled release. A cancer cell with a particular marker allows delivery of a pro-drug coupled to a co-delivered activating therapeutic gene. The

'ABCD' strategy [91] pioneered at Imperial College for use with siRNA recognises the problem of agent delivery; construction of an external shell around the siRNA allows it into the circulation, another to protects it from liver metabolism *etc*.

- Disadvantages
    - Network pharmacology dictates that the drug agent must act at multiple nodes without becoming non-selective. This challenge is highlighted in the example of beta adrenoceptors which have two major sub-types relevant to current pharmaceutical treatments – beta-1 which increase heart rate and force of cardiac contraction; and beta-2 which relax airways and the uterus and improve blood flow . The challenge with clinical use of beta-blockers is to aim to target multiple beta-adrenoceptor subtypes to ensure optimal effectiveness, while minimising ADR incidence e.g. from low cardiac output, including heart block. For cardiovascular treatment, cardio-selectivity is required to protect against heart attack without blocking beta-2 adrenoceptors in the lung to cause the adverse effect of bronchoconstriction.
    - Using a single drug entity means it is harder to titrate different doses based on individual functional variants.
    - Temporal or sequenced action requires innovative chemistry.
    - A single entity with sequentially evolved or designed multiple actions, is likely to be a large, complex molecule, bringing absorption, metabolism and excretion problems. A possible solution may lie in co-discovery and optimisation of multiple targets, which may require a move away from current single target discovery strategies.

Designing promiscuity into a small molecule is particularly challenging. 'Designed' multiple ligands (DMLs) [57, 92] have attempted to address this but tend to be larger and more lipophilic than conventional oral therapies. These molecules have lower ligand or binding efficiency [93] *i.e.* their binding energy per unit of molecular weight (MW) or lipophilicity (cLogP), which impacts on potency

and bioavailability of a compound. Dual ligands are generated by combining features of two selective ligands in a framework combination strategy. Success using DMLs was shown with the antipsychotic drug ziprasidone [94, 95] but the combination strategy can only be used when the two selective ligands are small and they can be well integrated. This poses a significant challenge to medicinal chemists and formulation scientists and some believe that development of a clinically effective single drug entity acting on different targets in different people is almost impossible. Medicinal chemistry literature has examples of molecules with recognised dual pharmacological properties, but they may not have progressed to therapeutics for several reasons. One may observe that ligands with polypharmacology including known toxic interactions may eventually achieve success (see pro-drug terfenadine above, redeveloped as fexofenodine [96]). However, in order to fully develop ligands exhibiting dual or polypharmacology, ensuring the clinical efficacy of both, or multiple targets in combination is necessary, which may be more costly and time-consuming. Failure using DMLs was seen with the antihypertensive drug omapatrilat [97] which was designed to inhibit both neutral endopeptidase 24.11 (NEP) and angiotensin converting enzyme (ACE) [98] as a putative solution to treating high blood pressure and heart failure [99]. Both enzymes are involved in the breakdown of bradykinin [100]. Their dual inhibition by omapatrilat precipitated serious adverse effects, including allergic type reactions (angio-oedema). Furthermore the observed efficacy of omapatrilat was less than predicted from the effects of separate monotherapies.

This example highlights the additional concern with single multi-targeted agents that promiscuity of action will increase off-target effects, thereby increasing ADRs and thus a network pharmacology approach to drug design may be seen as counter to personalized medicine. The use of multi-targeted therapeutics may in fact enable lower doses as multiple targets are modulated, provided there is an appropriate dose for the molecule with clinical efficacy without significant toxicity for each target. E nhanced knowledge of networks within a disease state will avoid inappropriate activation that might

cause ADRs. In many instances, combination therapy often has greater therapeutic selectivity than monotherapies thus additive ADRs may not necessarily result [101].

Formulation – a joint challenge for chemists and pharmacists.

Mixtures of monotherapies therefore appear promising as a pragmatic solution. As highlighted above, the main disadvantages relate to the formulation and release of the actives and to the design of clinical trials. These challenges are not in principle insurmountable. For example, fixed-dose combination (FDC) [102] drug products go some way to tackle the single monotherapy pill burden. These consist of the formulation of two or more active monotherapies combined in a single dose e.g. the antiretroviral drug Atripla® [103] contains three drugs – efavirenz, emtricitabine and tenofovir in one pill. Thus chemists as well as formulation scientists will play a role in personalising medicine in order to be able to find, harness and perfect formulations and combinations of drugs to maintain their bioavailability when combined in addition to altering titrations of each based on a network pharmacology analysis for perhaps different subsets of the population based on their heterogeneities and as such individualise therapy. This may actually be far more profitable for the pharmaceutical industry in that even if patents have expired on existing drugs, they could obtain the rights to sell the fixed-dose combinations known to be of therapeutic benefit.

Time release technology is an additional way in which formulations of drugs can be modified to allow the active ingredient to be released slowly over time [104]. Time release gives more sustained clinical benefits and improves adherence to treatment [105]. Using a personalized medicine approach, chemists could perfect formulations for the delivery of a drug allowing steady release to be achieved. Many of these temporal-control formulations rely on the expertise of numerous chemists and other scientists – some use matrixes of insoluble substances such as acrylics and chitin [106]. Polymer-based tablets have been produced with a small exit site for the drug on one side of the tablet and a porous membrane on the other side allows stomach acid to erode the membrane, thereby delivering the drug [107]. Further exciting developments using nanostructured delivery

agents[108] such as have been developed for several oncology therapeutics [109] continue with opportunities for input both from personalized medicine and network pharmacology agendas.

Formulation therefore becomes critical. For instance there has already been progress toward co-formulated products that can be prescribed to patients for those who do not achieve adequate glycemic control with metformin [110]. Physicians can prescribe a co-formulation of metformin with glyburide (Glucovance®) to increase insulin secretion from the pancreas, or co-formulated rosiglitazone and metformin (Avandamet®) aiming to improve insulin sensitivity [111]. In the context of ADRs it should be noted that following observation of increased cardiac events in patients treated with rosiglitazone [112, 113] a 'partial clinical hold' has been placed on a trial initiated by the FDA in 2007 [114]. In response to evidence of increased cardiovascular risk, the European Medicines Agency is due to announce in September 2010 whether rosiglitazone should remain available for selected lower risk patients or be withdrawn from clinical use. Chemists need to be aware of formulation and controlled release, alongside discovery of new monotherapies since these features are critically important in the personalized medicine paradigm as viewed in the new landscape of network pharmacology.

Better models, assays and screening tools are thus needed to aid multi-target drug discovery. Improved *in vitro* and *in silico* models are needed to demonstrate effectiveness of combination therapy and identify optimal experimental models [68]. Current cell-free assays inadequately model biological complexity and cell-based together with whole organism phenotypic assays are certainly more appropriate. Tumour cell lines used as proliferation assays, for measuring factors such as metabolic reduction or detecting apoptosis-associated cleavage reactions, are simple examples of such, as are broader phenotypic assays such as those with multiple cell types in order to probe a wider variety of disease-relevant networks due to higher levels of systems integration [61]. Using *in vivo* whole organism models such as the zebrafish [115] has been used to reveal further interactions.

Multi-target discovery

Chemistry is responding to the need for innovative screening technologies. Classic approaches with continued value in target identification include affinity chromatography of cell lysates [116] and geneticists' forward genomic approaches [117]. More recent developments in chemical genomics [118, 119] include photoimmobilisation and pull-down [120] yeast two-hybrid approaches [117] and protein complementation assays [29] that have been elegantly used to link therapies to 'hidden phenotypes' of drugs.

Previously unknown interactions between small, biologically active molecules and polypeptide "targets", have been uncovered in a phage-based chemical genomics approach, Magic Tag®, developed by researchers from the University of Warwick [121]. The method integrates phage display of polypeptides representing a proteome with photochemical immobilisation of bioactives on to a surface into different orientations to maximise binding from the polypeptide library [122, 123]. The technology has identified a potential interaction between β2-adrenoreceptor agonists such as salbutamol and nuclear hormone activating transcription factor 4 (ATF4) [121]. Although routinely used, these therapies are far from fully understood and hence chemical genomics approaches are ideal to help rationalise the molecular targets that a drug might bind and thus build up a biochemical picture of its mode of action. Informatic approaches such as recognition of shared side-effects [124] and chemical space descriptors of serious adverse drug reactions [125] are expanding rational network-aware approaches to the discovery of new targets and prevention of ADRs. Hence drugs that have been rejected in late stage clinical trials might also be examined to more fully understand reasons for observed toxicities or lack of efficacy thereby enabling re-purposing or personalized medicine approaches.

Conclusions

What is hoped for the field of personalized medicine[126] is to provide an improved diagnosis for patients, better treatment plans, modified drug discovery programs to integrate this knowledge and to expand preventative medicine. Completion of the Human Genome Project [127] and lists of associated gene products coupled with protein maps highlighting the complexity of pathways and interactions [128-130] has allowed pharmaceutical companies to effectively take a protein candidate from its cellular context and design highly specific drug compounds to antagonise or stimulate it. To what extent must chemists reappraise this drug discovery model?

Personalized medicine is clearly fuelling a major conceptual change within medicine. However, examples of personalized prescription of therapeutic drugs are relatively rare. The examples discussed above may highlight one of the reasons for this, since they are clear cases where variation in a single gene determines the outcome in the individual. The emerging concept of network pharmacology suggests such cases are rare and that the action of drugs is in general best understood as affecting a network. We suggest that major advances will be made when drug development programmes consider not only that drugs affect individuals differently, but also that drugs interact with multiple targets in each individual. Both paradigms consider that multiple variable targets are responsible for the diseased state and/or ADRs and we need to achieve a pleiotropic effect from small molecule drugs to act at these multiple, variable targets – moving away from the non-variable, single target drug discovery approach.

The central argument of this paper is that continued adherence to a single drug – single target paradigm will limit the ability of chemists to contribute to advances in personalized medicine whether they be in discovery or delivery. The way in which network pharmacology in particular will enable change highlights the need for greater interdisciplinary overlap between chemical biology, systems biology and clinical need. This new integrationist approach, accepting the idea of networks and nodes and the variation between them to create future therapies, invites chemists to play an

important role in their discovery and formulation. Implicit within this new view is the role played by educators in helping students approach these multifactorial problems.

Reductionism and the 'one-target-one-gene-one-disease' approach appear to have led to increased ADRs and fewer successful therapies [83] due to the lack of network pharmacology integration into the drug discovery process. An integrated network model [131] enabling chemists to chart multiple drug activity and their targets alongside undesirable off-targets [132] will advance drug development in both big and micro-pharma companies [133].

Future perspective

We conclude that small molecule drugs have a bright future in personalized medicine, often in combination therapies. However, the criteria used to design and select molecules for medicinal chemistry programmes may need to change to take greater account of the therapeutic delivery of the drug. Finally, the advent of tools that allow the discovery and manipulation of networks in a molecular fashion with improved prediction of individual response is likely to provide the sought-after leads.

Executive summary

The development of personalized medicine will further expand preventative medicine and individualized therapy in clinical settings.

- Chemistry and drug discovery can adapt in a number of ways by firstly appreciating that drugs affect individuals differently in addition to the need for them to modulate multiple targets in a network pharmacology framework.
- A shift is needed from a gene-centric to a network-centric view.
- Formulation and titration by chemists of new combinations of drug molecules will be key for future personalized drug prescription.
- Novel screening technologies will aid multi-targeted drug discovery.
- There is a need for a greater interdisciplinary approach to advance personalized medicine which includes clinicians prescribing, chemists and pharmacists formulating new combinations and medicinal chemists designing and discovering new therapies and delivery modalities.

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